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## PHASE DEVELOPMENT OF MICROBES AS AN OBJECTIVE REALITY

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[Comment: The following article from Mikrobiologiya was published as part of a discussion on noncellular forms of life being conducted by that periodical.

Numbers in parentheses refer to appended bibliography.7

A. S. Krivitski's recent article (1) represents the first attempt to refute a number of postulates advanced by V. Krestovnikova and myself. It is surprising that Krivitski identifies my hypothesis with the theories of G. Bosh'yan and M. D. Utenkov. Bosh'yan's inclusion of the phase of crystallization, in the cycle of development, his identification of filterable phases of bacteria with viruses, and Utenkov's assumption that phase development of microbes is based on a sexual process involving filterable zygotes are unnecessary complications of the very real and factual process of phase development.

The essential characteristics of the phase development of microorganisms which distinguish it from the phase development of higher organisms can be defined as follows: (a) beginning of the development from a noncellular form rather than from a cell; (b) the irregular manner in which the phases (stages) are passed and dependence of the periods of development on environmental factors; (c) the exceptional adaptability of young phases (stages) to external conditions; (d) the possibility that a phase (stage) may become stabilized for a prolonged period of time; and (e) interruption of the development at a certain phase (e. g., the granular phase may disintegrate, giving rise to the precellular phase, so that the development begins anew).

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Kriviskiy is incorrect in assuming that the ontogenesis of microbes consists of the development taking place between the time when a microbe population has been introduced into the nutrient medium and the time when it ages. Our concept of ontogenesis is much broader; we regard the phases of development as an alternation of types of assimilation and morphological structures which may include several generations (reseeds). Individual phases of development occasionally can be stabilized indefinitely. Kriviskiy confirms this himself by saying that he succeeded in preserving the granular phase and the microphase through an indefinite number of reseeds.

Kriviskiy seems to think that every microbial species must have its own type of phase development which is distinct from that of other species of microbes. It is difficult to agree with this: even at advanced stages of evolution, the phases of embryogenesis shown by different species are very similar or completely identical. The following phases of development have been postulated by us: the precellular (filterable), the granular (i. e., the microform), the coccal, and the bacillary. It seems to us that this subdivision accurately reflects the phylogenetic development of microbes, especially since the stages of this development have been actually observed by many investigators, including Kriviskiy.

Kriviskiy raises a pertinent question by asking whether one may assume a biological necessity for the formation of filterable forms either in the course of evolution or at present and whether these forms represent an obligatory stage in the development of microorganisms. One may definitely state that the filterable form is very resistant and essential for the survival of bacterial species, even though its resistance may be lower than that of spore forms. Kriviskiy's assertions that microorganisms remain in the vegetative state while they pass through thousands of generations and that they never have to assume the filterable form to be able to propagate from generation to generation under a great variety of external conditions are entirely erroneous. Filterable forms occur much more frequently and in much larger quantities than Kriviskiy believes. Early methods for the isolation of filterable forms yielded results in the same range as Kriviskiy's, but more recent work done with the aid of perfected techniques indicated that much larger quantities are present. My own results obtained on the phagolysate of *B. coli* were as follows:

The freshly prepared lysate was diluted in the ratio of  $10^{-12}$  with bouillon and placed into a constant temperature closet. One or 2 days later, further dilution in the ratios of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  resulted in material which showed growth of typical *B. coli*, which, however, were slimy and resistant to phage. Consequently, one may conclude that there was a secondary growth of phage-resistant forms. Within 10 days, a delicate growth appeared in the dilution  $10^{-8}$  and 2 days later in the dilution  $10^{-7}$ . The isolated cultures exhibited every typical property of secondary cultures, namely, polymorphism, forms which were initially granular, biochemical inactivity, and delicate, sluggish growth. The typical properties of *B. coli* could be restored only after a number of reseeds. By using a more efficient method of regeneration, it was also possible to isolate secondary cultures from the dilutions  $10^{-9}$ ,  $10^{-10}$ , and  $10^{-11}$ . Consequently, the content of precellular forms reached 100 billion units (i. e., particles) per one milliliter of phagolysate. Under the circumstances one cannot agree with Kriviskiy's statement that one unit of the filterable phase forms per 40-80 milliliters of the medium.

Kriviskiy is incorrect in asserting that the conditions which comprise suitable nutrition and which are created artificially in the laboratory to force microbes to pass through the stages of phase development cannot be encountered in nature. One must not forget that under natural conditions,

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microbes, as a rule, develop in a state of association with other microorganisms, and that for this reason V. V. Suknev's method of stimulating passage through the stages of phase development with the aid of "feeders" (2) is closest to the conditions existing in nature. The effect of these conditions is demonstrated by the presence in colonies and cultures of homologous "feeders" which constantly stimulate the transition of filterable forms into subsequent phases of development (i. e., daughter colonies and dwarf colonies).

Physiological disintegration due to autolysis begins under laboratory conditions after a culture has been grown for 24 hours and probably sets in still earlier under natural conditions. Kriviskiy's view that filterable forms do not form in the course of physiological disintegration must be designated as erroneous. Numerous experimentally established facts prove the presence of filterable forms in cultures 1-2 days old which are devoid of all traces of bacteriophage and have not been exposed to any of the forceful mechanical, physical, or biological influences which Kriviskiy regards as necessary for the formation of filterable forms. Kriviskiy is incorrect in assuming that microbial cells perish as a result of autolysis: their life is perpetuated by means of filterable forms.

In Kriviskiy's opinion, the changes of heredity often observed in connection with the regeneration of filterable forms furnish a convincing argument against the hypothesis of phase development. However, secondary cultures obtained as a result of passage through stages of the phase development often resembled the initial culture very closely. On the other hand, young phases exhibit a considerable amount of lability and adaptability which may result in a modification of inherited characteristics, but the fact that such modifications occur cannot be regarded as an argument against the existence of phase development. One must not forget that secondary cultures, which are formed as a result of the occurrence of phase development, come into existence under completely artificial conditions which do not correspond in the least to the natural requirements of the microbial species in question. However, the precise fact that young phases are capable of adapting themselves to unusual conditions indicates that our hypothesis is correct.

Numerous examples of the transformation of one microbial species into another, already known microbial species, which have been observed in the course of work on the regeneration of filterable forms, and which are of the greatest practical importance, exhibit one common trait: the new species which are formed always correspond to species that are phylogenetically older. Thus, dysentery bacilli of the Shiga type form bacilli of the Flexner type; Flexner bacilli form paratyphoid or coli bacilli; typhoid bacilli form microorganisms of the same species /paratyphoid or B. coli/; diphtheria bacilli form diphtheroid bacteria of the Hofmann type; and human tuberculosis bacilli form tuberculosis bacilli of the bovine or avian type. In prior work, we showed that as a result of vegetative hybridization in the intestine of dysentery patients, dysentery bacilli acquire properties which bring them close to B. coli or B. paracoli. It was found that the modified dysentery bacilli also acquire characteristics of Morgan's bacteria (formation of hydrogen sulfide, mobility, and gas formation in nutrient media containing glucose). Such "masked" strains can be reconverted into the initial dysentery bacilli: we succeeded in transforming a strain of Morgan's bacilli, the purity of which was established by the Grasse method, into Flexner dysentery bacilli via the filterable form. During the past 6 months, our laboratory /not identified/ has not been working with dysentery cultures and has not kept them in stock /so that accidental contamination could not have been assumed/.

When Kriviskiy identifies filterable forms of bacteria with viruses, he makes an error which is committed both by some adherents of the theory of phase development (Bosh'yan, Utenkov, and Crofton) and some of its opponents.

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However, the connection between some "viruses" and microorganisms which accompany them cannot be denied. Kriviskiy is incorrect in asserting that Krestovnikova's assumptions of the existence of a genetic connection between viruses and cellular microorganisms cannot be proved. It is reasonable to assume that viruses were derived from cellular forms in the course of evolution. Some viruses (e. g., the causative factors of neuroinfections, smallpox virus, and plant viruses) have lost entirely all recessive characteristics which connect them with their ancestral cellular forms, while others (e. g., the causative factors of hog cholera, scarlet fever, measles, influenza, and psittacosis) are invariably encountered together with certain bacteria in cases of infection. As far as the second type of viruses is concerned, Krestovnikova postulates in a sufficiently convincing manner the existence of a genetic connection between viruses and cellular forms. Kriviskiy's assertions, which are purely speculative, conflict with experimental data. Filterable forms which have been stabilized to such an extent that they may be regarded as "viruses" have lost all resemblance to ordinary, commonly encountered filterable forms. Under the circumstances, all arguments by which Kriviskiy tries to prove that there is a distinction between filterable forms and viruses become futile.

Investigations carried out by me in 1941 prove that filterable forms can be stabilized and do not change into subsequent stages (phases) in the course of a number of passages through animals. Work by E. Friedberger, V. Bisceglie, P. Giani, and F. Magrassi proves that pathogenic bacteria such as typhus bacilli and cholera vibrios, after they have been passed through animals, change into forms which resemble "viruses." The typical clinical form of the disease and formation of an immunity both toward the virus form and the initial bacterial form indicate the specific character of this process. Br. Fejgin succeeded in isolating secondary forms of cellular microorganisms after a number of passages through animals. However, Ph. Hadley and H. Carapetian were able to isolate secondary cultures only when regeneration was carried out outside the animal's organism, i. e., in vitro: seedings of material taken directly from the animals, being negative, produced the illusion that this material was sterile. Analogous phenomena, which resemble the results described above, were observed in work on typhus: cultures of proteus X could be isolated by Fejgin from the blood of patients only when passage through the stages of phase development was forced in the test tube. Similarly, isolation of the granular phase of proteus X could be achieved by Krestovnikova and co-workers only under these conditions.

As has been correctly stated by Kriviskiy, identification of bacteriophage with filterable forms is unjustified. Nevertheless, there can be no doubt that phages originated from filterable forms. Transformation of filterable forms into bacteriophage involves a change of qualitative characteristics and modification into another species. Consequently, the problem of intraspecies antagonism is irrelevant to this discussion. Although the newly formed phage loses its resemblance to the filterable forms from which it originated, it retains, as established by Krestovnikova, some of the antigenic properties of the filterable forms. In disputing the correctness of my theory, Kriviskiy cites data which pertain exclusively to lysogenic cultures, i. e., cultures which are phage-resistant. However, a number of investigators both in the USSR and abroad have demonstrated that aging phage-sensitive cultures develop bacteriophage. My own results show that a bouillon culture of a typical phage-sensitive Flexner bacilli strain develops bacteriophage within 9 days under conditions which preclude the possibility that the culture may have carried the phage in a latent state.

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